

CLAIMS

1. A method for purifying a carboxylated glycan, said method comprising:
 - a) providing:
 - i) a molecule comprising a carboxylated glycan;
 - ii) biotinylated diamino pyridine (BAP); and
 - iii) an exoglycosidase;
 - b) conjugating said molecule to said BAP to produce a BAP-glycan conjugate;
 - c) treating said BAP-glycan conjugate with said exoglycosidase to produce a first treated BAP-glycan conjugate comprising a first anionic BAP-glycan conjugate having from 1 to 2 negative charges per molecule; and
 - d) isolating said first anionic BAP-glycan conjugate, thereby purifying a carboxylated glycan.
2. The method of Claim 1, further comprising the steps of:
 - e) treating said first anionic BAP-glycan conjugate produced in step c) or step d) with an exoglycosidase to produce a second anionic treated BAP-glycan conjugate comprising a second anionic BAP-glycan conjugate having from 1 to 2 negative charges per molecule; and
 - f) isolating said second anionic BAP-glycan conjugate, thereby purifying a carboxylated glycan.
3. The method of Claim 2, further comprising repeating steps e) and f) from 1 to 10 times.
4. The method of Claim 1, wherein said molecule is a glycoprotein or polysaccharide.

5. The method of Claim 1, wherein said isolating comprises fractionating by ion exchange chromatography.

6. A method for purifying a carboxylated glycan, said method comprising:

- a) providing a molecule comprising a carboxylated glycan;
- b) isolating from said molecule a first anionic glycan containing from 1 to 4 negative charges; and
- c) desialylating said isolated first anionic glycan to produce a desialylated anionic glycan containing from 1 to 4 negative charges, thereby purifying a carboxylated glycan.

7. The method of Claim 6, further comprising d) isolating from said first desialylated anionic glycan a second anionic glycan containing from 1 to 4 negative charges, thereby purifying a carboxylated glycan.

8. The method of Claim 6, further comprising prior to step a) the step of treating said molecule with a proteinase enzyme.

9. A method for identifying a test agent as reducing specific binding of a polypeptide to a carboxylated glycan, comprising:

- a) providing:
 - i) a carboxylated glycan purified by the method of Claim 1;
 - ii) an antibody that specifically binds to said carboxylated glycan; and
 - iii) a test agent;
- b) contacting said purified carboxylated glycan, said antibody, and said test agent; and
- c) detecting a reduction in the level of binding of said antibody to said carboxylated glycan in the presence of said test agent compared to in the absence of said test agent, thereby identifying

said test agent as reducing specific binding of a polypeptide to a carboxylated glycan.

10. The method of Claim 9, further comprising d) identifying said test agent as reducing inflammation or cancer.

5 11. The method of Claim 9, wherein said purified carboxylated glycan is attached to a solid surface.

12. The method of Claim 11, wherein said carboxylated glycan attached to said solid surface is purified by the method of Claim 6.

10 13. The method of Claim 9, wherein said molecule comprising said carboxylated glycan is a glycoprotein or polysaccharide.

14. The method of Claim 13, wherein said molecule is a glycoprotein.

15. The method of Claim 14, wherein said glycoprotein is a receptor for advanced glycation end products (RAGE).

16. The method of Claim 15, wherein said antibody is monoclonal.

15 17. The method of Claim 16, wherein said monoclonal antibody is an IgG antibody.

18. The method of Claim 9, wherein said antibody is specific for a carboxylated glycan purified by the method of Claim 1.

19. The method of Claim 18, wherein said antibody is monoclonal.

20. The method of Claim 19, wherein said monoclonal antibody is an IgG antibody.

21. The method of Claim 20, wherein said monoclonal IgG antibody is mAbEE4.1, mAbGB3.1, mAbB2.6, or mAbEH2.7.

5 22. The method of Claim 20, wherein said monoclonal IgG antibody is mAbGB3.1.

23. A method for identifying a test agent as reducing specific binding of a polypeptide to a carboxylated glycan, comprising:

- 10 a) providing:
- i) a carboxylated glycan purified by the method of Claim 6;
 - ii) leukocyte cells; and
 - iii) a test agent;
- b) contacting said purified carboxylated glycan, said leukocyte cells, and said test agent; and
- 15 c) detecting a reduction in the level of adhesion of said leukocytes to said purified carboxylated glycan in the presence of said test agent compared to in the absence of said test agent, thereby identifying said test agent as reducing specific binding of a polypeptide to a carboxylated glycan.

20 24. The method of Claim 23, further comprising d) identifying said test agent as reducing inflammation or cancer.

25. The method of Claim 23, wherein said carboxylated glycan is attached to a solid surface.

26. The method of Claim 23, wherein said molecule comprising said carboxylated glycan is isolated from endothelial cells.

27. The method of Claim 23, wherein said molecule comprising said carboxylated glycan is a glycoprotein or polysaccharide.

5 28. The method of Claim 27, wherein said glycoprotein is a receptor for advanced glycation end products (RAGE).

29. A carboxylated glycan purified by the method of Claim 1.

30. The carboxylated glycan of Claim 29, wherein said molecule comprising said carboxylated glycan is a glycoprotein or polysaccharide.

10 31. The carboxylated glycan of Claim 30, wherein said glycoprotein is a receptor for advanced glycation end products (RAGE).

32. A carboxylated glycan purified by the method of Claim 6.

33. An antibody produced by EE4.1 cells, GB3.1 cells, B2.6 cells, or EH2.7 cells.

15 34. An antibody produced by GB3.1 cells.

35. An antibody specific for a carboxylated glycan purified by the method of Claim 1.

36. The antibody of Claim 35, wherein binding of said antibody to said carboxylated glycan is reduced by a carboxylated glycan, and said binding is not

reduced by a carboxylate-neutralized glycan selected from an alkyl esterified glycan or alkylamidated glycan.

37. The antibody of Claim 36, wherein said alkyl esterified glycan is CONH-methyl-glycan.

5 38. The antibody of Claim 36, wherein said alkylamidated glycan is a methylamidated glycan.

39. The antibody of Claim 38, wherein said antibody is monoclonal.

40. The antibody of Claim 39, wherein said monoclonal antibody is an IgG antibody.

10 41. The antibody of Claim 40, wherein said monoclonal IgG antibody is mAbGB3.1.

42. The antibody of Claim 35, wherein said antibody does not specifically bind to glucuronic acid, galacturonic acid, sialic acid, lactic acid, pyruvic acid, or uronic acid.

15 43. The antibody of Claim 35, wherein said antibody does not specifically bind to a sulfated glycan.

44. The antibody of Claim 43, wherein said sulfated glycan is contained in thyroglobulin or neural cell adhesion molecule (N-CAM).

20 45. The antibody of Claim 35, wherein said antibody does not specifically bind to a glycosaminoglycan.

46. The antibody of Claim 45, wherein said glycosaminoglycan is contained in chondrosamine, chondroitin sulfate, chondroitin sulfate tetramer, chondroitin sulfate octamer, hyaluronic acid tetramer, hyaluronic acid octamer, heparin, or heparan sulfate.

5 47. The antibody of Claim 35, wherein said antibody does not specifically bind to a phosphorylated sugar selected from the group consisting of glucose-1-phosphate, glucose-6-phosphate, mannose-6-phosphate, galactose-6-phosphate, glucose-N-acetyl-1-phosphate, and glucose-N-acetyl-6-phosphate.

10 48. The antibody of Claim 35, wherein said antibody does not specifically bind to a sulfated sugar selected from the group consisting of glucose-6-sulfate and galactose-6-sulfate.

49. The antibody of Claim 35, wherein said molecule is a glycoprotein or polysaccharide.

50. The antibody of Claim 49, wherein said glycoprotein is a receptor for advanced glycation end products (RAGE).

15 51. The antibody of Claim 50, wherein said antibody is monoclonal.

52. The antibody of Claim 51, wherein said monoclonal antibody is an IgG antibody.

53. A hybridoma cell line that produces a monoclonal antibody selected from the group consisting of mAbEE4.1, mAbGB3.1, mAbB2.6, and mAbEH2.7.

20 54. A hybridoma cell line that produces monoclonal antibody mAbGB3.1.

55. A method for reducing extravasation of leukocyte cells in endothelial tissue, comprising:

a) providing:

i) endothelial tissue comprising leukocyte cells; and

5 ii) an agent that reduces specific binding of a polypeptide to a carboxylated glycan purified by the method of Claim 1; and

10 b) administering said agent to said endothelial tissue such that specific binding of said polypeptide to said carboxylated glycan is reduced, thereby reducing extravasation of said leukocyte cells in said endothelial tissue.

56. The method of Claim 55, wherein said molecule comprising said carboxylated glycan is a glycoprotein or polysaccharide.

57. The method of Claim 56, wherein said glycoprotein is a receptor for advanced glycation end products (RAGE).

58. The method of Claim 55, wherein said polypeptide comprises S100A8, S100A9, S10012, amphoterin, annexin I, or amino acids 1 to 12 of annexin I.

59. The method of Claim 58, wherein said polypeptide comprises a S100A8•S100A9 heterodimer, (S100A8)₂•S100A9 heterotrimer, or
20 (S100A8)₂•(S100A9)₂ heterotetramer.

60. The method of Claim 58, wherein said polypeptide comprises S100A12.

61. The method of Claim 58, wherein said polypeptide comprises amino acids 1 to 12 of annexin I.

62. The method of Claim 61, wherein said polypeptide comprises amino acids 1 to 40 of annexin I.

63. The method of Claim 62, wherein said polypeptide comprises annexin I.

5 64. The method of Claim 58, wherein said polypeptide comprises amphoterin.

65. The method of Claim 55, wherein said agent is identified by the method of Claim 9.

66. The method of Claim 55, wherein said agent is identified by the method of Claim 23.

10 67. The method of Claim 55, wherein said agent is an antibody specific for said carboxylated glycan.

68. The method of Claim 67, wherein said antibody is monoclonal.

69. The method of Claim 68, wherein said monoclonal antibody is an IgG antibody.

15 70. The method of Claim 69, wherein said monoclonal IgG antibody is mAbEE4.1, mAbGB3.1, mAbB2.6, or mAbEH2.7.

71. The method of Claim 69, wherein said monoclonal IgG antibody is mAbGB3.1.

20 72. The method of Claim 55, wherein said agent is an anti-S100A8 antibody.

73. The method of Claim 55, wherein said agent is an anti-S100A9 antibody.

74. The method of Claim 55, wherein said agent is an anti-S100A12 antibody.

5 75. The method of Claim 55, wherein said agent is an anti-annexin I antibody.

76. The method of Claim 55, wherein said agent is an antibody specific for amino acids 1 to 12 of annexin I.

10 77. The method of Claim 55, wherein said agent is an antibody specific for amino acids 1 to 40 of annexin I.

78. The method of Claim 55, wherein said agent is an anti-amphoterin antibody.

79. A method for reducing adherence of leukocyte cells to endothelial cells, comprising:

- 15 a) providing:
- i) leukocyte cells;
 - ii) endothelial cells; and
 - iii) an agent that reduces specific binding of a polypeptide to a carboxylated glycan purified by the method of Claim 1;
- 20 and
- b) contacting said leukocyte cells, said endothelial cells, and said agent such that adherence of said leukocyte cells to said endothelial cells is reduced in the presence of said agent compared to in the absence of said agent.

80. The method of Claim 79, wherein said polypeptide comprises S100A8, S100A9, S10012, amphoterin, annexin I, or amino acids 1 to 12 of annexin I.

81. The method of Claim 79, wherein said agent is identified by the method of Claim 9.

5 82. The method of Claim 79, wherein said agent is identified by the method of Claim 23.

83. The method of Claim 79, wherein said agent is an antibody specific for said carboxylated glycan.

84. The method of Claim 83, wherein said antibody is monoclonal.

10 85. The method of Claim 84, wherein said monoclonal antibody is an IgG antibody.

86. The method of Claim 85, wherein said monoclonal IgG antibody is mAbEE4.1, mAbGB3.1, mAbB2.6, or mAbEH2.7.

15 87. The method of Claim 85, wherein said monoclonal IgG antibody is mAbGB3.1.

88. A method for reducing inflammation in a tissue in a mammalian subject, comprising:

a) providing:

- 20 i) a tissue; and
ii) an agent that reduces specific binding of a polypeptide to a carboxylated glycan purified by the method of Claim 1; and

- b) administering said agent to said tissue such that inflammation in said tissue is reduced in the presence of said agent compared to in the absence of said agent.

89. The method of Claim 88, wherein said mammalian subject is human.

5 90. The method of Claim 88, wherein said agent is identified by the method of Claim 9.

91. The method of Claim 88, wherein said agent is identified by the method of Claim 23.

10 92. The method of Claim 88, wherein said agent is an antibody specific for said carboxylated glycan.

93. The method of Claim 92, wherein said antibody is monoclonal.

94. The method of Claim 93, wherein said monoclonal antibody is an IgG antibody.

15 95. The method of Claim 94, wherein said monoclonal IgG antibody is mAbEE4.1, mAbGB3.1, mAbB2.6, or mAbEH2.7.

96. The method of Claim 94, wherein said monoclonal IgG antibody is mAbGB3.1.

97. The method of Claim 92, wherein said antibody is conjugated to a cytotoxin.

98. The method of Claim 92, wherein said antibody is conjugated to an imaging molecule.

99. The method of Claim 92, wherein said antibody is chimeric.

100. The method of Claim 89, wherein said administering is before
5 manifestation of inflammation in said tissue.

101. The method of Claim 89, wherein said administering is concomitant with manifestation of inflammation in said tissue.

102. The method of Claim 89, wherein said administering is after manifestation of inflammation in said tissue.

103. The method of Claim 89, wherein said human subject has or is
10 suspected of being capable of developing Crohn's disease, tumor growth, metastasis, diabetes, Alzheimer's disease, dementia, atherogenesis, periodontal disease, skin immune responses, septic shock, heart disease, arthritis, sarcoidosis, tuberculosis, chronic inflammation, or acute inflammation.

104. The method of Claim 89, wherein said human subject has or is
15 suspected of being capable of developing endotoxic shock, Crohn's disease, ulcerative colitis, multiple sclerosis, anaphylactic reaction, nephritis, asthma, conjunctivitis, systemic lupus erythematosus, ocular inflammation, allergy, emphysema, ischemia-reperfusion injury, fibromyalgia, psoriasis, rheumatoid arthritis, gouty
20 arthritis, juvenile rheumatoid arthritis, and osteoarthritis.

105. The method of Claim 89, wherein said human subject has or is suspected of being capable of developing Crohn's disease or ulcerative colitis.

106. The method of Claim 88, wherein said polypeptide comprises S100A8, S100A9, S10012, amphoterin, annexin I, or amino acids 1 to 12 of annexin I.

107. A method for reducing cancer in a mammalian subject, comprising:

a) providing:

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i) a mammalian subject; and

ii) an agent that reduces specific binding of a polypeptide to a carboxylated glycan purified by the method of Claim 1; and

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b) administering said agent to said subject such that cancer in said subject is reduced in the presence of said agent compared to in the absence of said agent.

108. The method of Claim 107, wherein said mammalian subject is human.

109. The method of Claim 107, wherein said agent is identified by the method of Claim 9.

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110. The method of Claim 107, wherein said agent is identified by the method of Claim 23.

111. The method of Claim 107, wherein said agent is an antibody specific for said carboxylated glycan.

112. The method of Claim 111, wherein said antibody is monoclonal.

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113. The method of Claim 112, wherein said monoclonal antibody is an IgG antibody.

114. The method of Claim 113, wherein said monoclonal IgG antibody is mAbEE4.1, mAbGB3.1, mAbB2.6, or mAbEH2.7.

115. The method of Claim 113, wherein said monoclonal IgG antibody is mAbGB3.1.

5 116. The method of Claim 112, wherein said antibody is conjugated to a cytotoxin.

117. The method of Claim 112, wherein said antibody is conjugated to an imaging molecule.

118. The method of Claim 112, wherein said antibody is chimeric.

10 119. The method of Claim 107, wherein said administering is before manifestation of cancer.

120. The method of Claim 107, wherein said administering is concomitant with manifestation of cancer.

15 121. The method of Claim 107, wherein said administering is after manifestation of cancer.

122. The method of Claim 107, wherein said polypeptide comprises S100A8, S100A9, S10012, amphoterin, annexin I, or amino acids 1 to 12 of annexin I.